MUTATIONS OF Pro30Leu AND Val281Leu OF THE CYP21 GENE IN PATIENTS DIAGNOSED WITH AMBIGUOUS GENITALIA

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ABSTRACT

Congenital adrenal hyperplasia (CAH) is a group of autosomal recessive disorders of adrenal steroidogenesis. The genes of the steroidogenic enzymes and the mutations involved have been described. Deficiency of the 21-hydroxylase (21-OH) enzyme is by far the most common form of CAH which arises as a result of deletions or deleterious mutations in the active gene (CYP21) located on chromosome 6p. Many different mutations of the CYP21 gene cause varying degrees of impairment of 21-OH activity that results in a spectrum of disease expression. There is no sharp limit between the salt- wasting, the simple virilizing and the late onset forms.

The objective of our study was to determine the 21-OH deficiency mutation defects and correlate the genotype with their phenotypic expression of the disease.

We performed mutational analysis using PCR-ASOH (Polymerase Chain Reaction - Allele Specific Oligonucleotide Hybridization) technique on six patients who presented with ambiguous genitalia (AG) and or electrolyte derangement as hyponatraemia and hyperkalaemia, suspected to have CAH. The Val281Leu and Pro30Leu mutations result in enzymes with 20-60% of normal activity and both are associated with the non-classical form of CAH. The Gln318stop mutation is categorized under the salt-wasting type.

Among the six patients, three had Val281Leu mutation, two had Pro30Leu mutation and one had Gln318stop mutation. The three patients with Val281Leu mutation had presented with adrenal crises during infancy and was classified as salt losers and treated with glucocorticoids and mineralocorticoids. These 3 patients could well be the other 40% who are categorized as salt-losers. The two patients with Pro30Leu mutations have normal male external genitalia and presented with hyponatraemia and hyperkalaemia. Only one patient required mineralocorticoid therapy that was given for about 5 months duration. Subsequently he had normal electrolytes level even without mineralocorticoid therapy. The Gln318stop mutation was identified in one patient who presented with ambiguous genitalia and adrenal crises.

Our study showed that the patients with genotype Val281Leu, Pro30Leu and Gln318stop mutations correlated with their phenotype. The mutation analysis of CYP21 gene proved to be a good complementary investigation and supportive to the diagnosis and management of our CAH patients.

Key words: ambiguous genitalia, congenital adrenal hyperplasia, CYP21 gene.

INTRODUCTION

Ambiguous genitalia (AG) are one of the clinical presentations of congenital adrenal hyperplasia (CAH). Congenital adrenal hyperplasia is an autosomal recessive disease caused by loss or severe decrease in the activity of 21-hydroxylase (21-OH). This enzyme is one of the five enzymes necessary for cortisol biosynthesis. Deficiency of the 21-OH is the most common form of CAH accounting for 90-95% of all cases of CAH[1,6].

Congenital adrenal hyperplasia presents a wide spectrum of clinical manifestations and patients are divided into 3 groups: salt wasting (SW), simple virilizing (SV) and non-classical (NC). Salt wasting patients manifest as neonatal electrolyte disturbances together with virilization of external genitalia at birth in girls and early pseudoprecocious puberty in boys, while SV patients present the same manifestation as SW patients, but without electrolyte disturbances. Non- classical patients present with late onset symptoms of androgen excess, ranging from progressive virilization and pseudoprecocious puberty in childhood to menstrual disturbances, infertility and hirsutism in adult women[2].

It was reported that more than 90% of cases of CAH are caused by mutation of the CYP21 gene. This 21-OH (CYP21) gene is located in the HLA class III gene region on chromosome 6p21.3 and consist of 10 exons[3]. Majority of the mutations on the CYP21 gene are Val281Leu and Pro30Leu. These mutations were reported to result in 20-60% of normal enzyme activity and both are associated with the non-classical form of CAH[4,5].

AIM OF THE STUDY

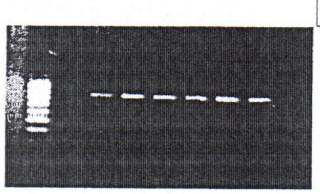
To detect the presence of point mutations in Pro30Leu, Ile172Asn & Val281Leu of CYP21 gene

MATERIALS AND METHODS

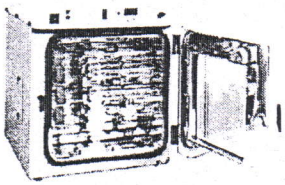
Blood samples were obtained from patients referred to Hospital Universiti Sains Malaysia, Kelantan, Malaysia during 1995-2002. A thorough clinical examination and hormonal analyses were performed. A total of 52 samples included were suspected to have CAH based on ambiguity of the external genitalia or electrolyte imbalances.

DNA extraction (non-phenol chloroform standard procedure)

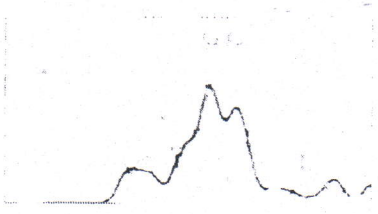
Polymerase Chain Reaction (PCR)



PCR-ASOH (Allele Specific Oligonucleotide Hybridization) mutational analysis using PCR-ASOH technique



DNA sequencing



RESULTS

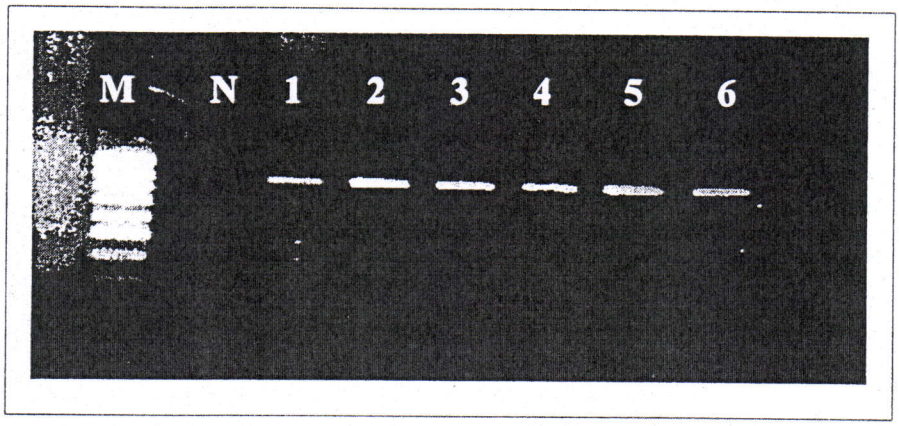


Figure 1: Presence of PCR product (873 bp) for CYP21 gene exon 1-3 using 2.0% agarose gel electrophoresis. Lane M : 100 bp DNA ladder, lane N : negative control, lane 1, 2 : normal samples, lane 3,4,5,6 : samples showing PCR amplification products for CYP21 gene.

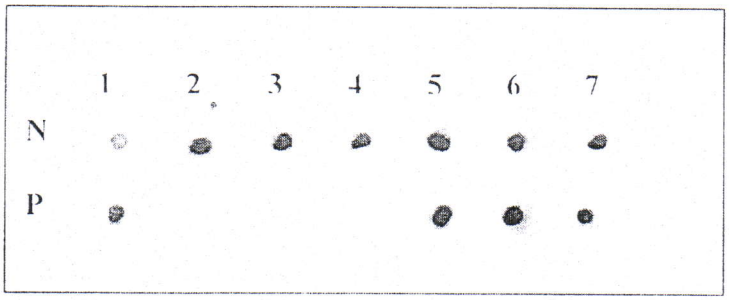


Figure 2: Dot blotting was performed using 1 µg genomic DNA. The blot was hybridized with 100 pmol/ml of the digoxigenin labeled antiphosphatase (DIG-AP) specific probe from samples of patient 1-7. ASOH of PCR product from CAH patients was performed with the probe corresponding to the site exon. The status N: normal, P: patient.

RESULTS

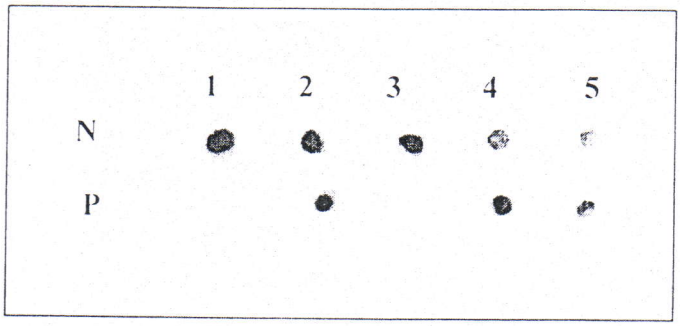


Figure 3: Pro30Leu hybridization DNA samples. Dot blotting was performed using 1 µg genomic DNA. The blot was hybridized with 100 pmol /ml of the digoxigenin labeled antiphosphatase (DIG-AP) specific probe Pro30Leu. ASOH of PCR product from CAH patients was performed with the probe (Pro30Leu) corresponding to the site exon 1. The status N: normal, P: patient.

RESULTS

Table 1

	Patient I	Patient II	Patient III	Patient IV	Patient V	Patient VI
Birth Weight (kg)	4.2	4.3	3.0	3.3	2.8	2.7
Age	32 days	81 days	2 months	At birth	10 weeks	6 days
Clinical Picture	Adrenal crisis Default follow up CPP Mental Retardation	Failure to thrive Normal male external genitalia Both testes descended Sepsis, meningitis & adrenal crisis	Haematuria Salt loss Normal male external genitalia Both testes descended	Normal male external genitalia Both testes descended Hypoglycae-mia	Failure to thrive Ambiguous genitalia Phallus 2 cm Single orifice at its base No palpable gonads	Neonatal jaundice Ambiguous genitalia Prominent phallus Single orifice at its base No palpable gonads Registered as male Reassigned as female
Sodium 135-150 mmol/L	120	110	119	131	92	118

RESULTS

	Patient I	Patient II	Patient III	Patient IV	Patient V	Patient VI
Potassium (3.5 – 5.0 mmol/L)	4.3	4.2	6.5	6.6	7.4	8.2
Blood urea (1.4 – 6.8 mmol/L)	28.8	23.0	NA	3.5	NA	NA
HCO3 (18 – 25 mmol/L)	1.6	4.4	NA	NA	NA	NA
17-OHP (ng/ml)	NA	NA	9.9 (0.07-1.7)	NA	>20 (up to 1.1)	33.2 (0.7-2.5)
Cortisol (nmol/L)	48 (139-501)	329 (138-690)	609 (140-500)	650 (138-690)	484 (139-501)	NA

RESULTS

	Patient I	Patient II	Patient III	Patient IV	Patient V	Patient VI
Testosterone (0.42-0.72 nmol/L)	1.4	NA	4.9	13.3	NA	NA
SRY gene	46,XY Present	46,XY Present	46,XY Present	46,XY Present	46,XX Not present	46,XX Not present
US pelvis / abdomen	-	-	-	-	Uterus & ovaries	Uterus & ovaries
Mutation in CYP21 gene	Val281Leu	Val281Leu	Pro30Leu	Pro30Leu	Val281Leu	Gln318Stop

NA: Not available, CPP: central precocious puberty

DISCUSSION

Majority of the mutations in the CYP21 gene in our patients are Val281Leu and Pro30Leu. These mutations were reported to result in 20-60% of normal enzyme activity and both are associated with the non-classical form of CAH[4,7].

In our study, we found that 3/52 patients (5.7%) have Val281Leu while 2/52 patients (3.8%) have Pro30Leu respectively. No mutations were observed in Ile172Asn. Patients with Val281Leu presented with adrenal crisis during infancy and were classified as salt wasting. Two patients with Pro30Leu mutations have normal male external genitalia and presented with hyponatraemia and hyperkalaemia. We did not identify any patient with I172N mutation which is known to result in clearly reduced enzymatic activity. About 1-2% of I172N mutation is usually associated with the SV form[8, 9].

Our findings showed that patients with Pro30Leu mutations were associated with non-classical form of CAH whereas Val281Leu mutations were associated with salt wasting form of CAH.

CONCLUSION

This study suggested that phenotypes are not always concordant with the genotype in patients with Val281Leu mutations diagnosed with CAH.

ACKNOWLEDGMENT

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Original Articles

Molecular Analysis in the management of Congenital Adrenal Hyperplasia (CAH) and Ambiguous Genitalia

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Patients presenting with ambiguous genitalia (AG) often pose a dilemma to the attending clinicians with regard to sex assignment. To avoid gender confusion in later life, it is important to recognize the underlying cause, initiate treatment and appropriate sex assignment as soon as possible. We present 3 case reports to illustrate the use of fluorescence in-situ hybridization (FISH), chromosome karyotyping and SRY gene identification in the evaluation of patients with AG. Current hormonal and imaging studies for sex determination could be complemented by the application of chromosomal and molecular genetic studies which provide rapid and reliable genetic diagnosis. Delay in diagnosis and treatment may result in death due to undiagnosed adrenal crisis in case of congenital adrenal hyperplasia (CAH) and wrong sex assignment with its tragic consequences. Molecular study and determination of the SRY gene are additional tools for the investigations of patients with AG and intersex disorders.

Key words: congenital adrenal hyperplasia (CAH), ambiguous genitalia (AG), sex determination region-Y (SRY).

INTRODUCTION

The issue of gender assignment causes anxiety and the problem of acceptance on the part of the parents. Patients with ambiguous genitalia should be investigated in order to understand the underlying pathology and to provide a rational approach to management and sex assignment. Diagnosis is aided by hormonal, imaging studies and genetic information. Current clinical and hormonal investigation protocols for sex determination can be enhanced by the application of chromosomal and molecular genetic studies using SRY gene.

The word ambiguous means indeterminate, doubtful or uncertain. The term ambiguous genitalia (AG) describe external genitalia that do not conform to that of male or female. This would include severe hypospadias with or without palpable gonads or micropenis[1]. Other description for such abnormalities include enlarged phallus/clitoris, scrotalisation of the labioscrotal folds, partial or complete fusion of the labioscrotal folds, chordee, urogenital sinus and blind vaginal pouch.

The problem in the management of a baby with AG is not only sex determination and appropriate assignment, it is also prompt recognition of underlying

medical emergencies that may be associated. The clinician must must be quick to detect life–threatening adrenal crisis in case of salt losing congenital adrenal hyperplasia (CAH)[2], and initiate immediate investigations and management[3,7]

The initial treatment goals include firstly, determination of the appropriate sex of rearing as quickly as possible. Secondly, establishment of definitive diagnosis, which may take longer to achieve. To avoid gender identification crisis, social, personality and religious problems during adulthood, follow up care and management is needed to ensure physical and psychological development concordant with the assigned sex[4].

Availability of specific hormonal investigation is limited. Conventionally, genetic sex is determined by chromosome karyotyping. The result is generally available within one month but a preliminary report may be available within a week. Currently new methods are available using molecular technology such as fluorescence in-situ hybridization (FISH) using the X (Figure 7) and Y (Figure 8) probes to identify the types of chromatin present. Identification of SRY gene (Figure 9) is another of these new molecular techniques [4].

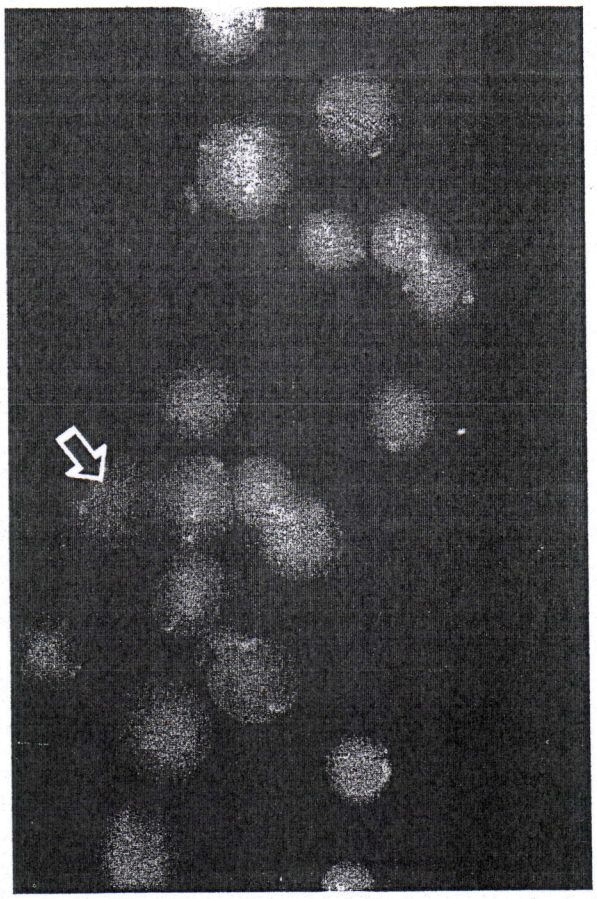

Figure 7: *Microphotography of FISH technique using the chromosome X probe (pDMX1) on interphase cell, arrow shows 2 signals of the chromosome X*

The SRY gene is the testis-determining gene located on the short arm of the Y chromosome. It initiates a cascade of events that ultimately lead to testis differentiation. Studies showed that SRY gene in an individual indicates the presence of testicular tissues. The use of the SRY gene testing as a tool in complementing hormonal, imaging studies in the evaluation of children with AG is helpful. The result can be obtained in less than a week [4]. Decision on gender assignment and registration of birth of baby can be made without much delay.

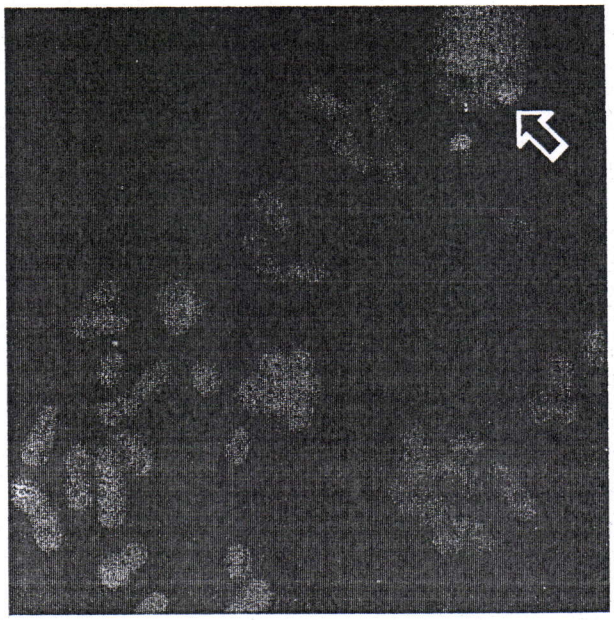

Figure 8: *Microphotography of FISH technique using pHY.1 probe. Arrow shows the specific Y chromosome.*

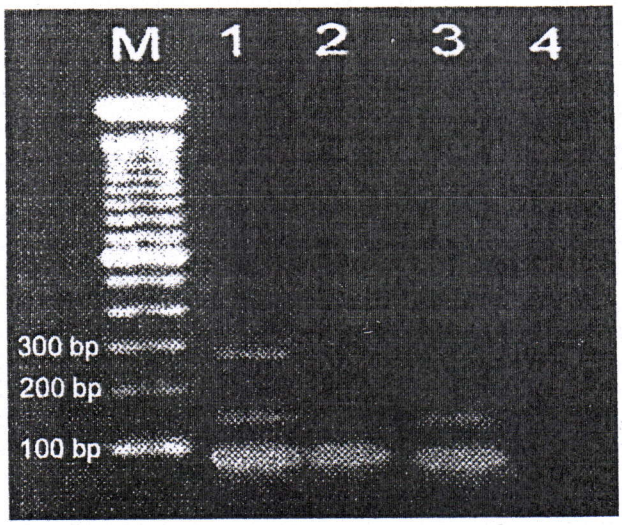

Figure 9: *Photograph of amplified multiplex PCR products using X, Y chromosome and SRY gene specific PCR primers.*
M- 100 bp DNA ladder (marker)
1 - DNA sample from 46 XY with the presence of SRY gene (273 bp)
2 - DNA sample from 46 XX in the absence of the SRY gene
3 - DNA sample from 46 XY with the absence of SRY gene
4 - Negative control (without DNA)

OBJECTIVE

The aim of the study is to apply the molecular studies to complement the investigations for the genetic assignment of patients with ambiguous genitalia by presenting 3 case reports.

METHODS

Three patients were referred to Hospital Kota Bharu (HKB) and Hospital Universiti Sains Malaysia (HUSM) for further management of ambiguous genitalia. Blood specimen of 3-5 ml from the patients with ambiguous genitalia was collected in sodium EDTA or lithium heparin container. Blood was cultured for metaphase cells for cytogenetic and FISH according to the standard protocol. Genomic DNA was extracted and subjected to the PCR analysis of SRY gene method[5, 6].

RESULTS

Patient 1, presented to Hospital Kota Bharu (HKB) at 10 weeks of age with intermittent vomiting and failure to thrive since birth. The patient, a product ofa non-consanguineous marriage was born full term by spontaneous vaginal delivery at a district hospital with a birth weight of 2.8 kg (25-50th percentile), length 56 cm (>97th percentile) and head circumference 33 cm (10-25th percentile). She is the youngest of 5 siblings and the eldest sibling who was a boy died at 17 days old of an unknown cause. On examination she was mildly dehydrated with a weight of 2.8 kg (<3rd percentile). She was afebrile and the blood pressure was 60/40 mmHg. No other abnormalities were detected apart from AG with a prominent phallus measuring 2 cm. No gonads or rugae were noted at the labioscrotal folds, which was not hyperpigmented. There was a single orifice at the base of the phallus. The baby was registered as a girl by the parents as they were not aware of the ambiguity of the external genitalia at that particular time.

The initial electrolytes showed hyponatraemia, hyperkalaemia, increased blood urea and metabolic acidosis (Table 1). A diagnosis of salt-losing CAH was made and she was started on glucocorticoids and mineralocorticoids. The hormonal investigations showed that 17-hydroxyprogesterone (17-OHP) and aldosterone were elevated with normal cortisol level (Table 1).

The results of hormonal investigation of the 3 patients are tabulated as follows:

Tests	Normal Range	Patient 1	Patient 2	Patient 3
Sodium	135-150 mmol/L	92	118	138*
Potassium	3.5 – 5.0 mmol/L	7.4	8.2	5.5*
Blood urea	1.4 – 6.8 mmol/L	11.0	NA	5.7*
Standard bicarbonate (HCO_3)	18 – 25 mmol/L	12.0	NA	NA
17-OHP		>20 ng/ml 3 mth-5 yr: up to 1.1 ng/ml	33.2 ng/ml (<0.7-2.5)	2.8 nmol/L** (normal for age)
Cortisol		484 nmol/L (am : 139-501)	NA	67 nmol/L* (am: 221-690 pm : 110-345)
ACTH		ND	50.7 pg/ml (N: <37)	ND
Testosterone		NA	<20 ng/dl (no reference)	3.0 nmol/L adult female: 0.9-2.8
Aldosterone		>3,300 pmol/L (111-860 pmol/L)	NA	1,400 pmol/L* erect: 110-860 supine : 30-440
Plasma renin activity		NA	NA	72.0 ng/ml/h* erect: 1.3-4.0 supine: 0.1-2.4
Karyotyping		46,XX	46,XX	46,XX
SRY gene		Not present	Not present	Not present
Ultrasound		Uterus and ovaries	Uterus	Uterus. Bilateral pelvi-calyceal system dilatation. Mildly distended bladder.
Genitogram		Figure 3	Figure 5	ND

*Table 1: **Results obtained prior to hormonal therapy * Patient was on hormonal therapy NA: not available ND: not done*

On follow-up she had normal weight gain and serum electrolytes. At 2 years of age there was evidence of under treatment as there was an acceleration in growth velocity. Bone age was reported as between 6 to 9 months old. The dose of cortisone acetate was increased to 10 mg/m^2/day and 5 months later, it was further increased to 30 mg/m^2/day. Serum 17-OHP was monitored. Her bone age at 6 years old was reported as 5 years and 10 months. The blood pressure was normal except for a borderline reading of 120/70 mm Hg at 4 6/12 and 5 6/12 years old.

At 6 5/12 years old, she was admitted to our centre for fever, vomiting and 3 episodes of fits. Patient had hypotension and needed assisted ventilation. She was managed as adrenal crisis and meningitis[1] (Figure 1). Her height was 110 cm (25-50th percentile), weight 20 kg (75-90th percentile). She appeared muscular with hypertrophied clitoris measuring 3.2 cm (Figure 2). During the course of admission she was noted to have hypertension that was initially attributed to the high dose of intravenous hydrocortisone.

An echocardiography performed revealed a thickened left ventricular wall with reduced contractility. Patient was thought to have a long-standing hypertension. She was started on antihypertensives and digoxin. Fludrocortisone was discontinued. The results of molecular studies showed a genotype of 46, XX and SRY gene was not present. Ultrasound of the abdomen and pelvis showed female internal reproductive system.

On follow-up, the serum electrolytes without mineralocorticoid therapy were normal. Genitogram of patient 1 (Figure 3) showed opacification of both the bladder and vagina. The perineal opening continued into a short segment urogenital sinus that bifurcated just at the level of inferior pubic rami. Clitoroplasty and vaginoplasty were performed. Patient was maintained on glucocorticoids at a dose of 16 mg/m^2/day and her blood pressure readings were normal.

Patient 2, was born full term by spontaneous vaginal delivery at a district hospital with a birth weight of 2.7 kg (25th percentile). He was registered as a boy at birth. On day 6 of life, he was referred to HKB for severe neonatal jaundice that required intensive phototherapy. Patient is the younger of 2 siblings and a product of a consanguineous marriage.

During hospitalization, it was noted that patient had an AG described as a prominent phallus, hyperpigmented labioscrotal folds with a single orifice at the base of the phallus and gonads were not palpable. Initial serum electrolytes showed severe

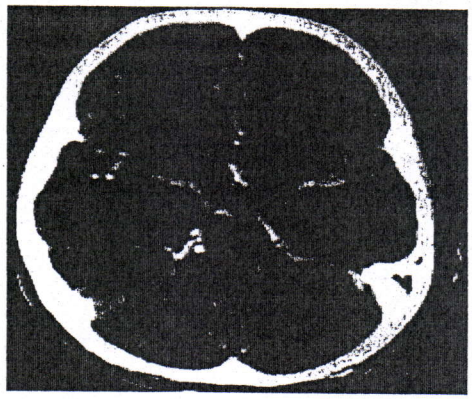

Figure 1: *CT scan of patient 1: Presence of meningeal enhancement on the right temporo-parietal region suggestive of meningitis.*

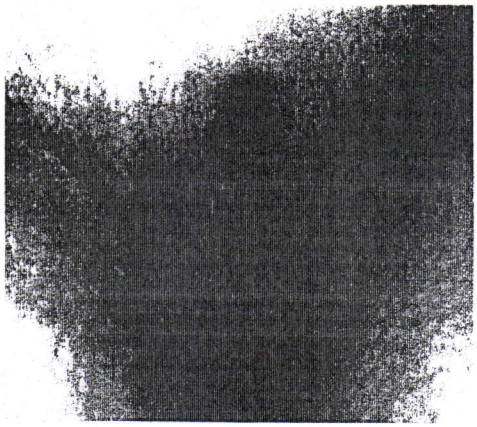

Figure 2: *External genitalia of patient 1: Enlarged clitoris of 3.2 cm.*

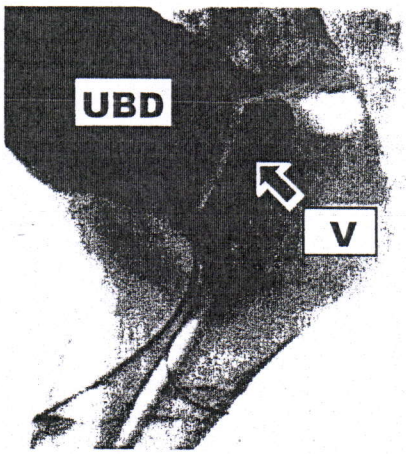

Figure 3: *Genitogram of patient 1: The urinary bladder (UBD) and vagina (V) are opacified with a single perineal opening.*

hyponatraemia and hyperkalaemia, Based on the clinical picture and electrolyte abnormality a diagnosis of CAH was made. Patient was started on glucocorticoid and mineralocorticoid. Hormonal

investigations showed elevated 17-OHP and ACTH level. The testosterone was <20 ng/dl. Chromosome analysis showed a 46, XX karyotype and SRY gene was not present. The ultrasound of abdomen and pelvis revealed the presence of a uterus (Figure 4) and the genitogram demonstrated a normal urinary bladder (Figure 5). Patient was re-assigned as female at the age of 6 months. Patient was first seen in our centre at the age of 9 months. Weight was 6.4 kg (10-25th percentile), height 64.3 cm (50th percentile) and head circumference 42.5cm (25-50th percentile). The external genitalia was hyperpigmented over the labia majora. Gonads were not palpable. The clitoris measured 2.5 cm with a single orifice at its base. Subsequent 17-OHP and testosterone level was suppressed while on treatment. Further surgical intervention was planned.

Patient 3 was referred to our centre on Day 13 of life for further management of AG. Patient was born full term by spontaneous vaginal delivery at a private hospital with a birth weight of 3.1 kg (75th percentile). The parents are non-consanguineous and patient has an elder sister who is healthy. Mother had a miscarriage for the first pregnancy. On Day 5 of life patient had hyponatraemia and hyperkalaemia. Based on the clinical finding of ambiguous genitalia and typical electrolyte changes, diagnosis of CAH was made. Treatment was started with glucocorticoids and mineralocorticoids after performing some hormonal investigations. Ultrasound of the abdomen revealed bilateral large adrenal glands and presence of a uterus.

On examination, the weight was 3.3 kg (75-90th percentile) and length 52 cm (>97th percentile). Baby had oral thrush and greenish discharge from the umbilicus. The external genitalia appeared feminine with a clitoris measuring 0.9 cm in length. There was slight hyperpigmentation and scrotalisation of the medial aspect of the labia majora. Gonads were not palpable. There was no fusion of the labia minora and there were 2 slit-like orifices seen representing the urethra and vaginal orifices. The electrolytes while patient was on hormonal therapy showed normal serum sodium and blood urea level with slight hyperkalaemia (Table 1).

Patient was maintained on glucocorticoids and mineralocorticoids pending results of hormonal investigations. A repeat ultrasound examination at our centre showed presence of uterus, normal suprarenal glands, dilatation of the pelvicalyceal system of both kidneys (Figure 6) and mildly distended urinary bladder. The urine examination was normal and no organisms were cultured. Umbilical swab showed a mixed growth of Gram negative organism and the full blood picture showed reactive thrombocytosis. The

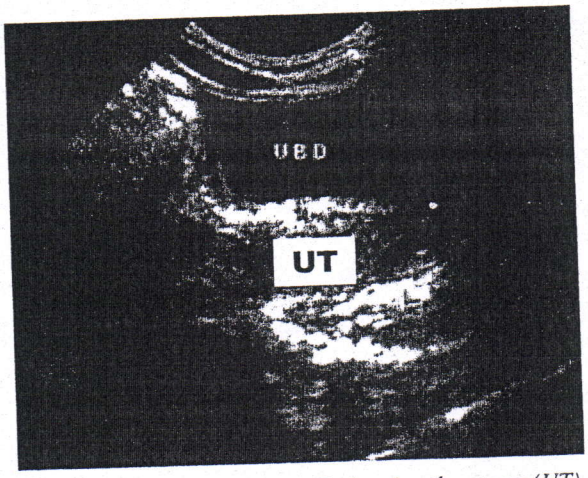

Figure 4, US pelvis of patient 2 showing the uterus (UT), urinary bladder (UBD)

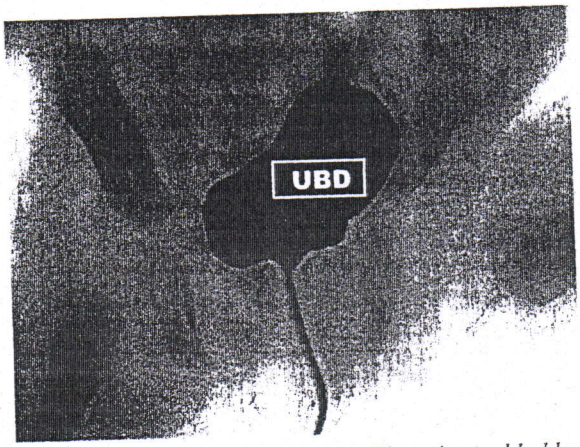

Figure 5, Genitogram of patient 2, The urinary bladder (UBD) was filled with contrast and normal in outline.

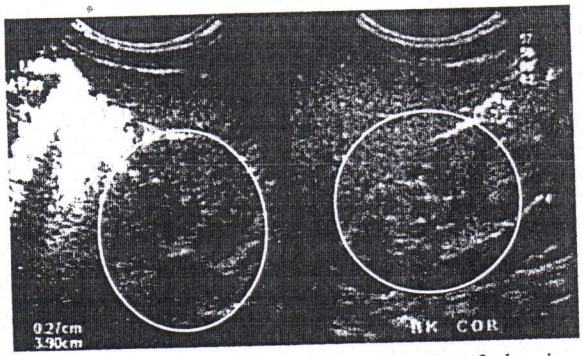

Figure 6, Ultrasound of the kidneys of patient 3 showing bilateral pelvicalyceal system dilatation (circle).

serum testosterone level was slightly elevated according to the adult female range. Serum 17-OHP level before commencement of hormonal therapy was normal. Aldosterone and plasma renin activity obtained when patient was on mineralocorticoid therapy were still elevated for age. Molecular studies confirmed that patient's karyotype was 46 XX, SRY gene was not present.

Based on the results of investigations, the glucocorticoids and mineralocorticoids were gradually tapered and discontinued respectively. On follow-up, the clitoris size normalized and the rugation of the medial aspect of the labia majora disappeared. Subsequent electrolytes values were within the normal range. A repeat ultrasound of the urinary system one month later was reported as normal.

DISCUSSION

The presentation of the 3 patients illustrates the problem encountered in managing AG/CAH. The diagnosis of CAH has to be suspected from the physical appearance, symptoms or positive family history. Investigations have to be done to confirm the diagnosis of CAH and the genetic sex of the child. Delay in diagnosis and treatment of CAH lead to the development of life threatening adrenal crisis as in patients 1 and 2. Wrong gender assignment has tragic consequences[8]. In a newborn baby with AG urgent investigations are warranted. Sex assignment should not be attempted based on appearance of external genitalia. Patient 2 was wrongly assigned as male and family had to suffer the psychological trauma of re-assignment as female at the age of 6 months. Genetic sex of the 3 patients were subsequently established using karyotyping and determination of SRY gene[9].

Congenital adrenal hyperplasia was suspected in patient 3 based on some ambiguity of the external genitalia and electrolyte imbalances at the initial presentation. After the relevant hormonal investigations were taken, patient was started on glucocorticoids and mineralocorticoids by the attending paediatrician so as to prevent possible adrenal crisis. There was elevated aldosterone level and plasma renin activity despite treatment. The full blood picture showed thrombocytosis and an umbilical swab showed a mixed growth of gram negative bacilli suggestive of possible infection. Urosepsis was ruled out from the urine examination and culture. In view of bilateral pelvicalyceal system dilatation and mildly distended urinary bladder, a vesicoureteric reflux and urinary tract infection were considered as possible causes. However we could not confirm vesicoureteric reflux on repeat ultrasound a month from presentation. Infection probably causes unresponsiveness of the distal renal tubules to aldosterone, a condition known as pseudohypoaldosteronism[10, 11].

Imaging studies as ultrasound (US), computed tomography (CT) scan and magnetic resonance image (MRI) of the abdomen/pelvis can aid in identifying the uterus (Figure 4) and gonads. The latter however may not be easily visualised. Genitogram needs to be done to identify the anatomy of the genitourinary system (Figure 3 and 5) that is important for future surgical correction of the external genitalia. Clitoroplasty is usually performed in infancy but not performed in patient 1 due to poor understanding of the condition by the parents. Vaginoplasty with division of the fused labial folds can be delayed until puberty.

Close regular evaluation and dosage adjustment of hormonal treatment especially glucocorticoids is needed for a successful treatment. Under dosage of glucocorticoids cause virilisation while over dosage causes hypertension (patient 1) and short stature as an adult.

CONCLUSION

Molecular studies for SRY gene is one of the tools for sex confirmation in addition to hormonal and imaging studies in the evaluation of AG. Determination of SRY gene may be helpful in sex determination and gender assignment. It is a rapid and reliable technique and will help the clinicians in making the right management decision. Regular medical assessment and monitoring of treatment is the key to the successful management of CAH/AG.

Molecular studies have enabled us to confirm the sex of the 3 patients presenting with AG. It has aided us in family counseling and further management including the surgical correction of the external genitalia as in patient 1 and 2. Furthermore we could counsel the parents with regards to sex reassignment in our second patient and prevent future virilisation and gender crisis. As for patient 3, we were able to counsel the parents who are anxious about the true gender of their baby and the fact that she need not receive life-long hormonal therapy. In the 3 patients that we managed, the parents were content to have the problem rectified, better understanding of the condition and to accept the appropriate management.

In our experience with the cases of AG, we noted that hormonal investigations should be done to confirm CAH and to include the molecular analyses as a complementary tool in the battery of investigations.

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